C). Clarence
#9
7.3.01

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

Y. HIGUCHI ET AL. : GROUP ART UNIT: 1743

SERIAL NO.: 09/473,165 :

FILED: DECEMBER 28, 1999 : EXAMINER: CROSS, L.

FOR: DRY MEASURING TEST DEVICE

DECLARATION UNDER 37 C.F.R. §1.132

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231
SIR;

I, Yoshihiko Higuchi, a citizen of Japan, one of the inventors of the above-identified application, of 4-18-15 Seikadai, Seika-machi, Soraku-gun, Kyoto-fu, Japan, hereby declare and state that:

1. I received a Bachelor of Engineering degree in 1989 from the Department of Applied Chemistry, Faculty of Engineering, Osaka Institute of Technology.

2. I have been employed by Arkray, Inc. (former Kyoto Daiichi Kagaku Co., Ltd.) since March, 1989.

3. I am engaged in research on development of test strips for clinical chemistry.

4. The following experiments were conducted by me or under my direct supervision.

Methods and results:

[Preparation of dry measuring test device]

I. Dry measuring test device 1 (Present invention)

Components for a reagent layer were mixed according to the composition of the dry measuring test device 1 shown in the following table to prepare a coating liquid for the reagent layer. Cell Guard (Hoechst Cellanese) was attached on a glass palte so

as to form no wrinkle. The coating liquid for the reagent layer was applied to the resulting Cell Guard by using a knife coater at a thickness of 100 μm and dried at 25°C and at a humidity of 15% for 30 minutes to form a reagent layer. Further, components for a light blocking layer were mixed according to the composition of the dry measuring test device 1 shown in the following table were mixed to prepare a coating liquid for the light blocking layer. The coating liquid for the light blocking layer was applied to the reagent layer by using a knife coater at a thickness of 40 μm and dried at 25°C and at a humidity of 15% for 30 minutes to form a light blocking layer. Then the Cell Guard, on which the reagent layer on which the light blocking layer was laminated ("light blocking layer/reagent layer) was provided, was peeled from the glass plate and cut into the size of 7 mm x 7 mm. The resulting light blocking layer/reagent layer on Cell Guard having a size of 7 mm x 7 mm square was attached by heat press on a PET film having a size of 30 mm x 7 mm with a hole of a diameter of 4 mm so that the side of Cell Guard could face the PET film to cover the hole. A cover of a thermoplastic resin was attached on the light blocking layer/reagent layer side of the PET film so as to form the capillary compartment between the cover and the PET film. The cover had a liquid sample-supplying hole and an air hole. Thus, a dry measuring test device of which light blocking layer contains polymer beads embedding carbon black (dry measuring test device 1) was prepared.

II. Dry measuring test device 2 (Comparative Experiment)

A dry measuring test device was prepar d in the same manner as the dry measuring test device 1 except that components for a reagent layer and a light blocking layer were mixed according to the compositions of the dry measuring test device 2 shown in the following table. Thus, a dry measuring test device in which carbon black were directly distributed in the layer (dry measuring test device 2) was prepared.

	Dry measuring test device 1 (Present invention)		Dry measuring test device 2 (Comparative experiment)	
	Reagent layer	Light block-ing layer	Reagent layer	Light block-ing layer
Borate buffer (150 mM, pH 7.0)	29.0 g	-	29.0 g	-
Hydroxypropyl cellulose	1.3 g	1.3 g	1.3 g	1.3 g
Techpolymer MBX-5 (White)*1	5.0 g	-	-	-
Techpolymer MBX-5 (Black)*2	-	5.0 g	-	-
Titanium dioxide particles	-	-	2.5 g	-
Carbon black	-	-	-	2.5 g
Propiofan (BASF)	1.3 g	1.3 g	1.3 g	1.3 g
TES buffer (300 mM, pH 7.0)	5.0 g	5.0 g	5.0 g	5.0 g
Tween-20 (50 wt%)	3.2 g	3.2 g	3.2 g	3.2 g
Glucose oxidase	138 kU	-	138 kU	-
Peroxidase	103 kU	-	103 kU	-
4-Aminoantipyrine	0.2 g	-	0.2 g	-
MAOS (Dojin)	0.5 g	-	0.5 g	-
Distilled water	2.4 g	38.6 g	2.4 g	38.6 g

*1 manufactured by Sekisui Kaseihin Kogyo; content of titanium dioxide: 50 wt%

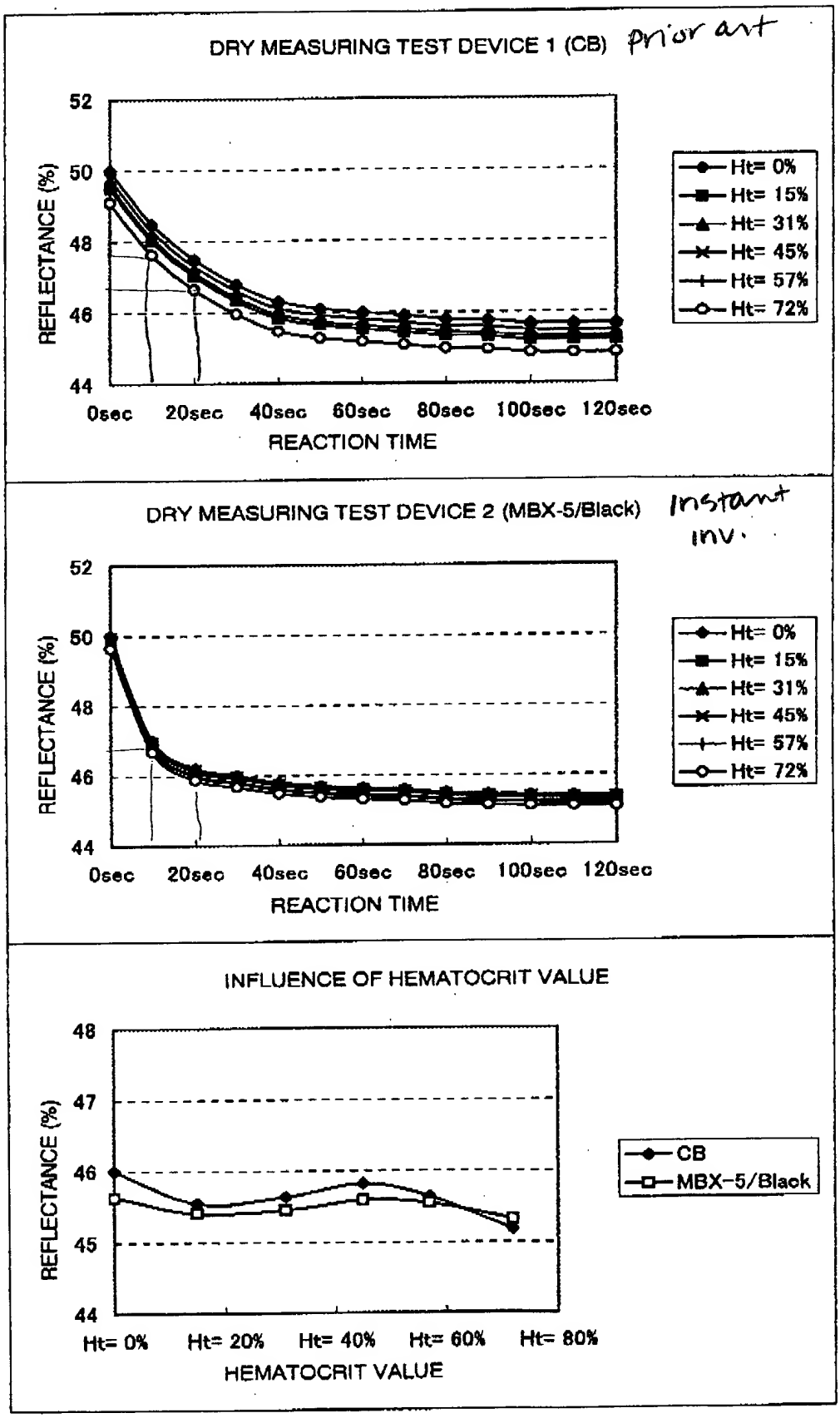
*2 manufactured by Sekisui Kaseihin Kogyo; content of carbon black: 50 wt%

The compositions were adjusted so that content of carbon black in the light blocking layer became the same between the dry measuring test devices 1 and 2.

[Evaluation of dry measuring test device]

The dry measuring test devices prepared in the above were tested for influence of the difference of the hematocrit value of the whole blood sample on the measured value.

Whole blood (glucose concentration: 99 mg/dl) supplemented with a glycolysis inhibitor (NaF) was adjusted to have different hematocrit values (0%, 15%, 31%, 46%, 57% and 71%) to prepare six samples. 10 μl of each sample was spotted on the reagent layer of the dry measuring test device of the present invention. From 5 seconds later, light of 640 nm was irradiated from the side of Cell Guard through the measuring light-irradiation hole and obtained reflectance was measured using a reflectiometer (color-difference meter). The same test was carried out for the comparative dry measuring test device.
The results are shown below.


DRY MEASURING TEST DEVICE 1 (CB)
prior art
REFLECTANCE (%)
52
50
48
46
44
0sec 20sec 40sec 60sec 80sec 100sec 120sec
REACTION TIME
Ht= 0%
Ht= 15%
Ht= 31%
Ht= 45%
Ht= 57%
Ht= 72%
DRY MEASURING TEST DEVICE 2 (MBX-5/Black)
instant inv.
REFLECTANCE (%)
52
50
48
46
44
0sec 20sec 40sec 60sec 80sec 100sec 120sec
REACTION TIME
Ht= 0%
Ht= 15%
Ht= 31%
Ht= 45%
Ht= 57%
Ht= 72%
INFLUENCE OF HEMATOCRIT VALUE
REFLECTANCE (%)
48
47
46
45
44
Ht= 0% Ht= 20% Ht= 40% Ht= 60% Ht= 80%
HEMATOCRIT VALUE
CB
MBX-5/Black

The upper graph shows the time-cource of reflectance in the measurement using the dry measuring test device 2 in which carbon black were directly distributed in the light blocking layer. At any of hematocrit values, the refelectance gradually decreased. This indicates that sinking of the sample into the reagent layer is slow.

The middle graph shows the time-cource of reflectance in the measurement using the dry measuring test device 1 in which polymer beads embedding carbon black were contained in the light blocking layer. At any of hematocrit values, the reflectance sharply decreased at the beginning. This indicates that sinking of the sample into the reagent layer is fast, in other words, the reaction proceeds fast. Because the rection proceeds fast, it is possible to shorten a measurment time.

The lower graph shows comparison of reflectance at 60 seconds between the dry measuring test devices 1 and 2. When polymer beads embedding carbon black was used, influence of hematocrit values on reflectance became small, compared with when carbon black was directly used.

Conclusion

By using polymer beads embedding carbon black, a measurement can be shortened and influence of hematocrit values on reflectance becomes small, whereby measurement can be made rapidly and accurately, compared with the direct use of carbon black.

I hereby declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: June 15, 2001

Yoshihiko Higuchi

Yoshihiko Higuchi